Phytochemical Screening on the Seeds of *Treculia africana* and *Artocarpus atilis*

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Abstract: *Treculia africana* Decne and *Artocarpus atilis* are highly valued economic plants and the seeds known for their numerous nutritional and medicinal potentials. In this study, a phytochemical analysis was conducted on the seeds of the two plants to reveal the phytochemicals in them. The results of the screening showed that the seeds contained phytochemicals such as alkaloids, flavonoids, saponins, tannins and cyanogenic glucosides (HCN). There was no detection of steroid in both samples. The alkaloid content of *Treculia Africana* was 0.12±0.02% and 0.08±0.02% for *Artocarpus atilis*. Saponin, flavonoid and tannin contents were 0.27±0.03%, 0.16±0.02% and 0.22±0.01% for *Treculia Africana* respectively and 0.33±0.03%, 0.18±0.02% and 0.19±0 for *Artocarpus atilis* respectively. Oxalate and hydrogen cyanide contents of *Treculia Africana* were 0.13±0.01 and 6.96±0.05% with *Artocarpus atilis* having 0.17±0.01 and 37.69±28.08 respectively. This indicates that both seeds are safe for human consumption.
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1. Introduction

Plants offer a large range of natural compounds belonging to different molecular families which have various properties to humans. These molecules possess interesting biological activities which attracted several researchers to their elucidation to provide knowledge that will lead to advancement medicine (Hervé *et al*, 2008). Phytochemicals are present in a variety of plants utilized as important components of both human and animal diets. These include fruits, seeds, herbs, and vegetables. They are organic substances that are found and accumulated by plants. According to Stampfer and Rimm (1993), phytochemicals are subsets of functional foods and are chemicals that are found in plants and plant derived foods.

Leonardo (2005) reported that phytochemicals are usually used to refer to compounds found in plants that are not required for normal functioning of the body but that nonetheless have a beneficial effect on health or play an active role on the amelioration of diseases thus they differ from what are traditionally termed nutrients in that, they are not necessary for normal metabolism and their absence will not result in a deficiency disease (Vivekananthan, 1995).

Treculia africana Decne and *Artocarpus atilis* belong to the family Moraceae and are native to many parts of West and tropical Africa. They grow commonly in evergreen and deciduous forests, often by streams but may sometimes be planted as in Nigeria where it is very common in the Western and Eastern states (Hutchinson, 1973). The family are monoecious or dioecious trees, shrubs, lianas, or rarely herbs comprising 40 genera and 1,000 species, nearly with milky sap. The leaves are simple and alternative or rarely opposite. The stipules are small and lateral or sometimes they form a cap over the bud and leave a cylindrical scan. The flowers are unisexual and minute, and are usually densely aggregated. These aggregations frequently take the form of pendulous aments or catkins.

Treculia africana Decne and *Artocarpus atilis* locally called *Ukwa* in Igbo vernacular is one of the most cherished economic plants, and is also a highly valued medicinal plant widely utilized in most preparations in the traditional herbal medicine. The seeds are a veritable source of highly nutritious *ukwa* food which is found to have an excellent polyvalent dietetic value and have also biological value of its proteins exceeding that of soybean (Enibe, 2006). The seeds are variously ccoked as porridge or roasted and sold with palm kernel (Edet *et al*, 1985). The flour has high potential usage for pastries. The seeds highly nutritious and constitute a cheap source of vitamins, minerals, proteins, carbohydrates and fats (Okafor and Okolo, 1974; Makinde and Elemo, 1985). Ethno-medicinally, *Treculia africana* is used as a vermifuge, febrifuge galactogogue and laxative (Irvine, 1961). The plant is also an important component of some ancient anti-diabetic recipe used in Western and Middle Belt of Nigeria. The crude extracts from different parts of the plant have been used in the folk medicine in the treatment of various

ailments. It is used either singly or in combination with other herbs in the traditional herbal preparations by different communities to treat various diseases. Decoctions from different plant parts are used as an anti-inflammatory agent and in the treatment of whooping cough. The crushed leaves juice is applied on the tongue as a treatment for thrush in children; the latex is applied as an antibacterial agent in eardrops, and as chewing stick.

The *T. africana* leaves decoctions were reported used in Trinidad and Bahamas to lower blood pressure (Morton, 1987), and is used also in some communities as an effective treatment in stomach upset and other gastro intestinal infections. Most of these claimed uses are yet to be scientifically verified and evaluated.

Inspite of the numerous nutritional and medicinal potential of the *Treculia africana* and *Artocarpus atilis* fruit, there is little analytical information on the chemical profile of the fruit and seed hence a phytochemical analysis was conducted on the fruits of the two plant species to support their use for food and feed formulation and give their nutritional values.

2. Material and Methods

Collection and Preparation of Plant Samples: The seeds of *Treculia africana* and *Artocarpus atilis* were collected from the Horticulture Unit of National Root Crops Research Institute Umudike. The seeds were dehulled, dried with oven at 43^{O}c and was ground into uniform powder using Thomas Wiltey milling machine.

Qualitative Phytochemical screening:
Phytochemical tests were carried out first to establish the presence or otherwise of some specific phytochemicals. The chemical tests were carried out with the standard specimens using standard procedures to identify the constituents as described by the Harbone (1988) and Sofowara (1993). The seeds were screened for alkaloids, saponins, tannins, flavonoids, phenols, steroids and terpernoids.

Quantitative Determination of the Phytochemicals
Determination of Alkaloid: 5g of each sample were analyzed in accordance with the alkaline precipitation gravimetric method (Harbone, 1973). The weighed sample was soaked in 100mls of 10% acetic acid solution of ethanol. The mixture was allowed to stand at room temperature for 4 hrs before it was filtered through Whatman filter paper. The filtrate was reduced to its original volume by evaporation over a steam bath. Alkaloid in the extract was precipitated by drop wise addition of concentrated NH_4OH until full turbidity was obtained. The precipitate was recovered by filtration using a previously weighed filter paper. The precipitate was then washed with 1% NH_4OH solution, dried in the oven at 100^{O}C for an hour. It was cooled in a desicator and reweighed. By difference the weight of alkaloid was determined and expressed as a percentage of the sample analyzed.

$$\% \text{ Alkaloid} = \frac{W_2 - W_1}{\text{Weight of sample}} \times \frac{100}{1}$$

Where W_1 = Weight of empty filter paper;
W_2 = Weight of paper + alkaloid precipitate

Determination of Flavonoids: The ethyl acetate precipitation method was used (Bohm and Kocipai, 1994). A weighed sample 5g was hydrolyzed by boiling in 100mls of 2mls of hydrochloric acid solution for about 35 mins. The hydrolysate was filtered to recover the extract (filtrate). The filtrate was treated with ethyl acetate drop wise twice until in excess. The precipitated flavonoid was recovered by filtration using a weighed filter paper after drying in the oven at 100^{O}c for 30 mins, it was cooled in a desiccators and reweighed. The difference in weighed gave the weighed of flavonoid which was expressed as a percentage of the weighed of sample analyzed.

$$\% \text{ Flavonoid} = \frac{W_2 - W_1}{\text{Weight of sample}} \times \frac{100}{1}$$

Where W_1 = Weight of empty filter paper;
W_2 = Weight of paper + flavonoid precipitate

Determination of Tannins: The Follins-Dennis spectrophotometric method (Pearson, 1976) was used. 1g of the dry test sample was dispensed in 50mls of distilled water and shaken for 30 mins in the shaker. The mixture was filtered and the filtrate was used for the experiment. 5mls of the extract was measured into 50mls volumetric flask and diluted with 35mls of distilled water. Similarly, 5mls of standard tannic acid solution and 5mls of distilled water were measured with separate flasks to serve as standard and blank respectively. These were also diluted with 35mls of distilled water separately. 1ml of Follins-Dennis reagent was added to each of the flasks followed by 2.5mls of saturated sodium carbonate solution. The content of each flask was made up with distilled water and incubated for 90mins at room temperature. The absorbance of the developed colour was measured at 760nm wavelength with the reagent blank at zero. The experiment was repeated two more times to get an average.

$$\% \text{ tannin} = \frac{100}{W} \times \frac{Au}{As} \times \frac{C}{1000} \times \frac{Vf}{Va} \times D$$

Where W = Weight of sample analyzed;

Au = Absorbence of the test sample
As = Absorbance of standard tannic solution;
C = Concentration of standard I mg/ml;
Vf = Volume of filtrate analyzed
D = Dilution factor where applicable.

Determination of Saponin: Saponin content of the sample was determined by double solvent extraction gravimetric method (Harbone, 1973). 2g of the powdered sample was mixed with 50mls of 20% aqueous ethanol solution. The mixture was heated with periodic agitation in water bath for 90 mins at $55^{O}C$. It was filtered through filter paper through Whatman filter paper. The residue was extracted with 50mls of the 20% ethanol and both extracts were pooled together. The combined extract was reduced to about 40mls at $90^{O}C$ and transferred to a separating funnel where 40mls of diethyl ether was added and shaken vigorously. Separation was by partition during which the ether layer was discarded and the aqueous layer reserved. Re-extraction by partition was done repeatedly until the aqueous layer become clear in colour. The saponins were extracted with 60mls of normal butanol. The combined extracts were washed with 5% aqueous NaCl solution and evaporated to dryness in a pre-weighed evaporating dish. It was dried at $60^{O}c$ in the oven and reweighed. The experiment was repeated two more times to get an average.

$$\% \text{ Saponins} = \frac{W_2 - W_1}{\text{Weight of sample}} \times \frac{100}{1}$$

Where W_1 = Weight of evaporating dish;
W_2 = Weight of dish + sample

Determination of Phenols: The Follins method described by pearson (1979) was used to determine the phenol content. 0.2g of the dried sample was dispensed into a test tube. 10mls of methanol was then added to it and shaken thoroughly. The mixture was left to stand for 5 minute before being filtered using Whatman filter paper. Iml of the extract was placed in a test tube and 1ml of Follins reagent was added to it with 5ml of distilled water. The colour was allowed to develop for about 3 to 4 hrs at room temperature. The absorbence of the developed colour was measured at 760nm. The experiment was repeated two or more times to get an average. The phenol content was calculated as

$$\% \text{ Phenol} = \frac{100}{W} \times \frac{Au}{As} \times \frac{C}{1000} \times \frac{Vf}{Va} \times D$$

Where W = weight of sample analyzed;
Au = Absorbance of the test sample;
As = Absorbance of standard solution
C = Concentration of standard in mg/ml;
Vf = Total filtrate volume;
Va = Volume of filtrate analyzed
D = Dilution factor where applicable

3. Results

The results of the qualitative phytochemical screening on the two plant species, *Treculia africana* and *Artocarpus atilis* (tables 1) shows the presence of some important phytochemicals. From the result, it was found that the seeds contained phytochemicals such as alkaloids, flavonoids, saponins, tannins and cyanogenic glucosides (HCN). There was no detection of steroid in both samples.

Table 1: Qualitative phytochemical analysis of *Treculia Africana* and *Artocarpus atilis*

Samples	Alkaloid	Flavonoid	Tannin	Saponin	HCN	Steroid
Treculia africana	+ve	+ve	+ve	+ve	+ve	-ve
Artocarpus atilis	+ve	+ve	+ve	+ve	+ve	-ve

Table 2 below shows the result of the quantitative phytochemical analysis of the two plant species studied. The alkaloid content of *Treculia Africana* was 0.12±0.02% and 0.08±0.02% for *Artocarpus atilis*. Saponin, flavonoid and tannin contents were 0.27±0.03%, 0.16±0.02% and 0.22±0.01% for *Treculia Africana* respectively and 0.33±0.03%, 0.18±0.02% and 0.19±0 for *Artocarpus atilis* respectively. Oxalate and hydrogen cyanide contents of *Treculia Africana* were 0.13±0.01 and

6.96±0.05% with *Artocarpus atilis* having 0.17±0.01 and 37.69±28.08 respectively indicating that that oxalate and hydrogen cyanide are more in *Artocarpus atilis* than in *Treculia africana*.

Table 2: Quantitative phytochemical analysis of *Treculia Africana* and *Artocarpus atilis*

Samples	% Alkaloid	% Flavonoid	% Tannin	% Saponin	% HCN (mg/kg)	% Oxalate
Treculia africana	0.12±0.02	0.16±0.02	0.22±0.01	0.27±0.03	6.96±0.05	0.13±0.01
Artocarpus atilis	0.08±0.02	0.18±0.02	0.19±0	0.33±0.03	37.96±28.08	0.17±0.01

4. Discussions

The results of this study showed that the seeds contained phytochemicals, some of which are known to be nutritionally beneficial while others have some antinutritional activities. Flavonoids were found in both samples and are known to possess antioxidant activity. This implies that consumers of the seeds may be protected from the oxidative cell destruction by the antioxidant. On the other hand, alkaloids are often reported to be toxic and are able to initiate strong physiological changes in the body when consumed (Harbone, 1973). Tannins and cyanogenic glycosides are both aninutrients. Cyanogenic glocosides are substances which on hydrolysis, yield hydrocyanic acid-a known toxic substances to man and animals. The HCN level of *Treculia africana* was 6.96±0.05mg/kg oxalate much lower than the 36 mg/100 gDM considered being lethal to man. However a higher HCN level of 37.96±28.08mg/kg was observed in *Artocarpus atilis* thus indicating that it is unfit for consumption by man and animals The oxalate level was also low in both samples and unlikely to pose toxicity problems in food since it is far below the toxic levels of (Munro and Bassir, 1969; Oke, 1996). Excess consumption of oxalic acid can cause corrosive gastroenteritis (Fasset, 1973; Eastwood, 1986). The saponin content of the samples were 0.27±0.03% and 0.33±0.03% for *Treculia Africana* and *Artocarpus atilis* respectively. Ijeh (2004) reported that saponin is known to have hypocholesterolemie properties and as such, consumers of saponin containing seeds may enjoy chemoprotection against heart diseases.

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